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Original Article

RFLP clusters of rifampicin resistant and susceptible *Mycobacterium tuberculosis* strains in Western province of Sri Lanka

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Abstract

Introduction: Continuous studies on genetic diversity of *Mycobacterium tuberculosis* could enhance the awareness on transmission, control and prevention of tuberculosis (TB). In this study, we investigated current genetic diversity of TB and rifampicin resistant TB by, Restriction Fragment Length Polymorphism (RFLP) based on fingerprinting of the IS6110 insertion sequence, in the Western province of Sri Lanka, the famous touristic destination with the highest TB burden in the country.

Methodology: Genomic DNA extracted from susceptible and rifampicin resistant TB strains (confirmed for *rpoB* gene point mutations) were digested with *Pvu*II restriction enzyme, electrophoresed and subjected to Southern transfer. The blots were hybridised with IS6110 probe and visualized using a chemiluminescence detection.

Results: The number of copies of IS6110 per isolate varied from 1 to 14. The dendrogram revealed a total of 68 distinct strains among 77 TB isolates and they belonged to nine clusters. Both rifampicin resistant and susceptible strains were distributed in all clusters. This evaluation revealed the absence of genetically identical or strong relatedness between susceptible and resistant isolates. However, clonal expansion was detected in transmission of both TB and rifampicin resistant TB. In addition, the resistant isolates having the novel mutation had no clonal relatedness.

Conclusion: This is the first observational study regarding clonal expansion of TB in Sri Lanka. Thus, further investigation on genotypes, clonal expansion and transmission of drug resistance using additional markers would be useful for controlling TB.

Key words: Restriction fragment length polymorphism; *Mycobacterium tuberculosis*; rifampicin; Sri Lanka.

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Introduction

Mycobacterium tuberculosis is a causative agent of human tuberculosis (TB) that already infected one third of the world's population. Drug resistance is one of the major challenges in control and prevention of TB. Every year, around 440,000 multidrug resistant tuberculosis (MDR-TB) cases are isolated while around 150,000 persons are estimated to die because of MDR-TB [1]. MDR-TB mostly affect developing countries where little is known about the characteristics of the circulating drug resistant strains. As the genotype of the *M. tuberculosis* may vary between different geographical regions, it is possible to identify by DNA fingerprinting unreported *M. tuberculosis* strains with specific characteristics in different areas. Better knowledge of epidemiological situation of TB caused by different *M. tuberculosis* genotypes could help in revealing the source of infection and in tracking transmission routes of various strains [2]. Therefore, routine surveillance of molecular epidemiology of MDR-TB is essential to prevent and control drug resistant TB [3]. There are various methods for DNA fingerprinting of *M. tuberculosis* and for molecular epidemiology research. Known repeated sequences in *Mycobacterium* genome, such as insertion sequences (*IS*), trinucleotide repeats, variable number tandem repeats (VNRT), mycobacterial interspersed repetitive units (MIRU) and the direct repeat (DR) regions are commonly used as genetic markers for genotyping [4]. A high degree of DNA polymorphism in *M. tuberculosis* strains is associated with *IS*. Member of the *IS*3 family, *IS6110*, is the most abundant *IS* and play an important role in genome plasticity [5]. Restriction fragment length polymorphism (RFLP), based on the variable number of *IS6110* copies, is used as the genetic marker for molecular epidemiology of TB and especially *M. tuberculosis* strains, that may contain 0-25 *IS6110* copies [6]. RFLP analysis of *IS6110* is

considered as the standard strain-typing technique for *M. tuberculosis* due to its apparent mobility, average copy number, and high discriminatory potential in population epidemiological analyses [4].

In Sri Lanka, there are very little data on epidemiological characteristics of *M. tuberculosis* strains. Existing data do not consider clustering of *M. tuberculosis* strains and genetic relationship between drug resistant and susceptible strains. The present study aimed to investigate genetic diversity of rifampicin-resistant and -sensitive *M. tuberculosis* strains using RFLP fingerprinting in the Western province of Sri Lanka where a high TB incidence is reported. This is the first study that compares genetic polymorphism of rifampicin-resistant and rifampicin-sensitive *M. tuberculosis* strains in Sri Lanka.

Methodology

Isolation of M. tuberculosis cultures and study population

Sputum samples from patients suspected to have TB were collected from Chest Hospital Walisara and Central Chest Clinic Colombo, Sri Lanka from March 2008 to September 2010. Sputum samples were processed using the modified Petroff's method [7]. Decontaminated samples were inoculated on Lowenstein-Jensen (L-J) medium (Difco, Franklin Lakes, USA) and incubated at 37°C. Drug susceptibility testing was carried out using agar proportion method following CLSI guidelines [8]. Phenotypically and genotypically confirmed 31 rifampicin-resistant *M. tuberculosis* isolates and phenotypically confirmed 46 rifampicin-sensitive *M. tuberculosis* isolates were used for the RFLP analysis. H37Rv was used as the control strain.

Whole genome digestion and Southern transfer

Genomic DNA was extracted from selected *M. tuberculosis* strains by phenol chloroform extraction method. Restriction enzyme digestion was performed according to the method previously described, in a total volume of 100 µL by sequential addition of PCR grade water, 10 µL of 10X restriction buffer, 6 µg of DNA and 30 units of *Pvu*II restriction enzyme (Promega, Medison, USA) [9]. The mixture was vortexed and incubated at 37°C for 16 hours. The *Pvu*II enzyme was heat inactivated by incubating the mixture at 65°C for 10 minutes. The digested DNA (8 µL) was mixed with the loading buffer (4 µL) (Promega, Medison, USA) and was it separated by electrophoresis on ethidium bromide stained 1% agarose gel at 1.5 V/cm for 16 hours and visualized under the UV light.

The remaining digested DNA (92 µL) was precipitated by adding 9 µL sodium acetate (pH 5.2) (Sigma, St. Luis, USA) and 300 µL ice cold absolute ethanol (Sigma, St. Luis, USA), and was re-dissolved in 1X loading buffer (volume was determined according to the concentrations of DNA presence on the test gel) containing the molecular weight maker X (Roche diagnostics, Indianapolis, USA). The DNA mixture was allowed to dissolve overnight and separated on 0.8% agarose gel by electrophoresis at 2 V/cm for 16 hours.

Electrophoresed DNA was denatured by immersing the inverted gel in 500 mL denaturing buffer (1.5 mol NaCl and 0.5 mol NaOH) at 25°C for 30 minutes with shaking. The denatured gel was neutralized in 500 mL neutralizing buffer (1.5 mol NaCl and 0.4 mol Tris-HCl) at 25°C for 30 minutes with gentle shaking. The nylon membrane (GE healthcare, Little Chalfont, United Kingdom) was marked by spotting with orientation marker (mix of H37RV DNA and marker X (GE healthcare, Little Chalfont, United Kingdom) at 6 different positions on upper and lower margins of the membrane. The membrane was hydrated in 500 mL sterile distilled water for 30 seconds and equilibrated in 500 mL 20X SSPE (Saline-Sodium Phosphate-EDTA) buffer for 5 minutes. Then, Southern transfer was carried out for 16 hours. Thereafter, the Southern transfer set up was dismantled and the nylon membrane was put at 80°C for 2 hours in an oven and stored at 4°C.

Preparation and labelling of IS6110 probe

A 240bp fragment of *IS6110* insertion sequence of *M. tuberculosis* was PCR amplified to be used as the probe. The amplified product was purified using PCR amplification clean-up kit (Promega, Madison, USA) as per manufacture's guidelines. DNA concentration of the amplified *IS6110* fragment was determined using a NanoDrop (2000c/2000) UV-Vis Spectrophotometers (Thermo Scientific, Leicestershire, USA). For labelling, 200 ng of the probe was mixed with 13 µl PCR grade H_2O and heated at 100°C for 5 minutes followed by snap cooling on ice (4°C) for another 5 minutes. Thereafter, 15 µL of horseradish peroxidase and 15 µL of gluteraldehyde (GE healthcare, Little Chalfont, United Kingdom) were added sequentially. Finally, the mixture was incubated at 37°C for 10 minutes.

Probe hybridization and detection

Pre-hybridization of Southern blot was carried out in 48 mL ECL Gold buffer (GE healthcare, Little Chalfont, United Kingdom,) at 42°C for 1 hour in a

closed plastic bag. Thereafter, 45 µL of labelled probe was added directly to the Gold buffer (48 mL) in the closed plastic bag. Hybridization was carried out at 42°C for 16 hours with shaking. The hybridized membrane was washed twice in 400 mL washing buffer for 20 minutes at 42°C. Then, the membrane was washed twice in 400 mL of 2 X SSC (Saline Sodium Citrate) buffer at room temperature (~25°C) for 5 minutes with shaking. Finally, the membrane was put in a new plastic bag and incubated with 8 mL (4 mL of reagent one + 4 mL of reagent two) of Amersham ECL detection reagents (GE healthcare, Little Chalfont, United Kingdom) for 90 seconds. Thereafter, all the fluid was removed and the bag was sealed. An X-ray film was exposed to the membrane in a dark room for 5 - 10 minutes and the film was developed using standard methods. After completing detection, membrane was de-probed by incubation in 400 mL of boiling Sodium dodecyl sulphate (SDS) at room temperature (~25°C) for 1 hour with shaking. Thereafter, the membrane was put in a new plastic bag to carry out the hybridization with molecular weight maker X DNA (GE healthcare, Little Chalfont, United Kingdom). The probe labelling, hybridization and detection for maker X were carried out by similar methods as mentioned above. Finally, membrane was de-probed once again and stored at refrigerated conditions.

Analysis of rpoB gene mutations

Selected fragments of *rpoB* gene of rifampicin resistant isolates were amplified using designed specific primers. PCR products were purified and analysed by DNA sequencing [10].

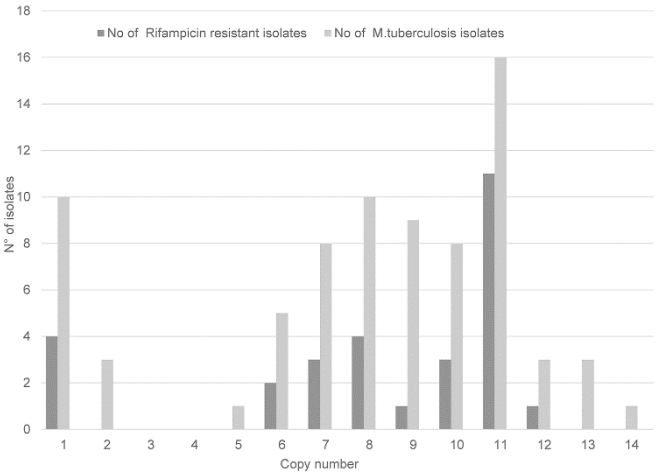
Analysis of data

The internal marker, marker X was used to estimate the sizes of *IS6110* fragments of RFLP fragments. The quality control of the procedure was done by comparing it with the *IS6110* fingerprint pattern of the reference strain, H37Rv. Genetic relationship among 77 isolates was analysed by the presence (1) or absence (0) of specific DNA bands of each isolate compared to the molecular weight marker X on the same lane. A distances matrix was computed in PHYLIP (version 3.69, Washington University) by the program Restdist (using the distance matrix), and internal branching probabilities were determined by bootstrap analysis using 100 replications. Cluster analysis was carried out with the same program using Neighbour Joining method (PHYLIP version 3.69, University of Washington, Washington, USA) and the dendogram was constructed using Phyfi software [11].

Results

IS6110 fingerprints for both strain categories (rifampicin-resistant or rifampicin-sensitive) were highly variable in respect to the number of copies and location of bands. The number of copies of *IS6110* per isolate varied from 1 to 14, with sizes range from 665-10180 bp. All isolates had at least one copy of the *IS6110* element. Copy number 11 was presented by most of tested isolates (n = 16, 20.8%). Within them, 11 strains were resistant to rifampicin. Isolates containing 3 or 4 copies were not present and isolates with copy numbers 2, 5,13 and 14 were found only among the rifampicin -sensitive strains (Figure 1). The genetic relatedness among *IS6110* fingerprint patterns were analysed using the distance matrix method and the Neighbour Joining dendrogram as shown in Figure 2. A total of 68 distinct patterns were found among 77 *M. tuberculosis* isolates. They were separated into two groups, G1 and G2 at node A. G1 was the larger group consisting 7 clusters (clusters 3- 9) where G2 had only two clusters (cluster 1 and 2). All clusters contained both rifampicin-resistant and rifampicin-sensitive strains. In addition, one isolate (M060) branched out independently. Isolate C432 was very closely associated with the H7Rv isolate (Wild type), at the root of the dendrogram. Cluster 5 included 87% of sensitive isolates and cluster 1 contained 83% resistant isolates. A large number of isolates (n = 15) were observed in cluster 8 and showed more distinct genetic relatedness with its lineages. Genetic identity or close relatedness between sensitive and resistant isolates was not observed in the dendrogram constructed according to the fingerprinting pattern of *IS6110* insertion elements (Figure 3). Among the five rifampicin-resistant isolates

Figure 1. *IS6110* copy numbers present in all *M. tuberculosis* isolates (n = 77) and only rifampicin-resistant *M. tuberculosis* isolates (n = 31).



in cluster 1, four isolates (C008, C010, C020 and C120) had identical fingerprints (Figure 2). The rifampicin-sensitive isolate (C424-branch length 0.01591) in cluster 1 was distinctly separated from all rifampicin-resistant isolates. Neighbor Joining dendrogram constructed for 31 rifampicin-resistant *M. tuberculosis* isolates (Figure 3) contains three clusters (cluster 1, 2 and 3). Nearly half of the rifampicin-resistant isolates (n = 13) were categorized into cluster 2. Two isolates were distinct and one of these (C006) was more related to the H37RV (wild type, branch length 0.01084) (Figure 3). Cluster 1 contained 3 genetically identical isolates (C008, C020, and C010). Further, in cluster 3, one clade contained two genetically identical isolates (C115 and C022) while its sister clade also had identical isolates (C027 and C007).

Mutations of *rpoB* gene of rifampicin resistant isolates are shown in the Table 1. Rifampicin resistant isolates with the novel mutation at codon 626 (n = 13) had 11distinct *IS6110* RFLP patterns and they were distributed in all clusters (Figure 3). Among them, C020 was genetically identical to C008 while the isolate C007

Figure 2. Rooted Neighbor - joining dendrogram constructed from *IS6110* RFLP fingerprinting patterns of *M. tuberculosis* isolates.

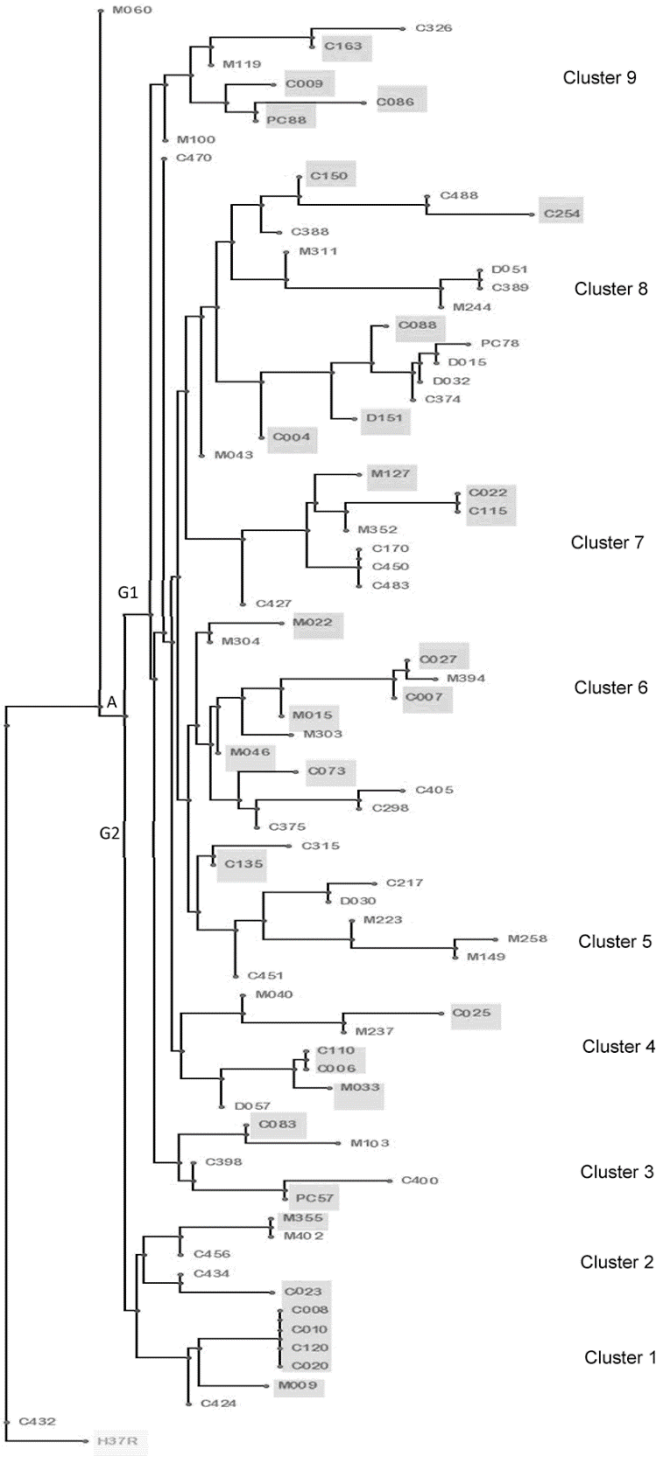


Figure 3. Rooted Neighbor - joining dendrogram constructed from *IS6110* RFLP fingerprinting patterns of rifampicin-resistant *M. tuberculosis* isolates.

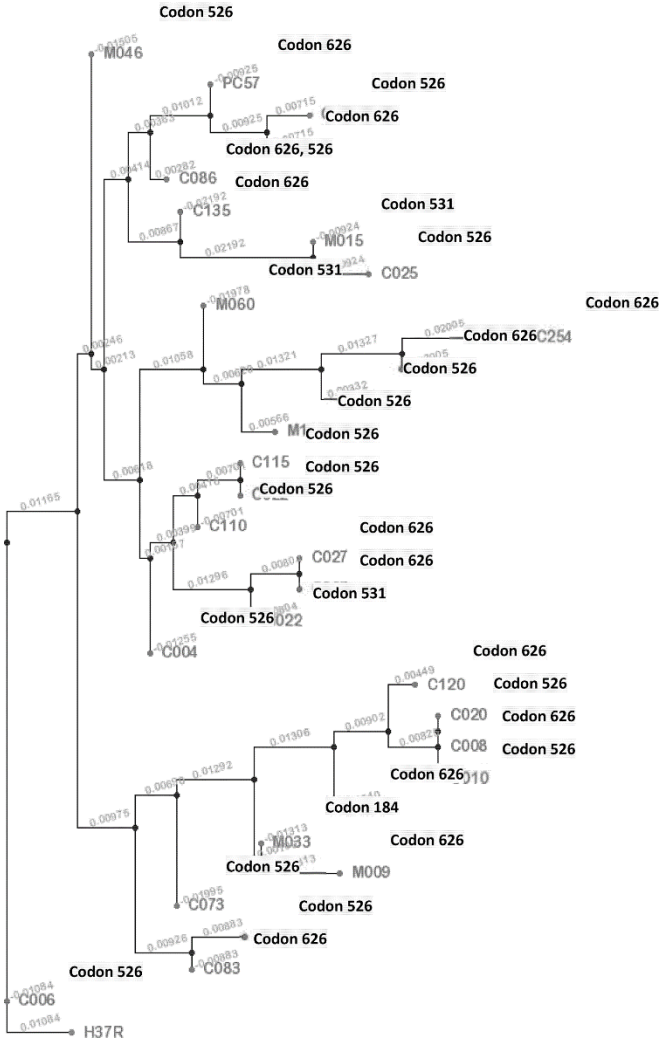


Table 1. Mutation types and frequencies observed for the rpoB gene of M. tuberculosis isolates in western province of Sri Lanka.

Mutated codon	Specific mutation	Percentage %
526*	CAC (His) → TAC (Tyr)	45.2
626$^{\$+}$	GAC(Asp) → GAG (Glu)	35.5
531*	TCG (Ser) → TTG (Leu)	9.7
184$^{\$+}$	GAC(Asp) → GAT(Asp)	3.2
626,184	GAC → GAG,GAC → GAT	3.2
526, 626	CAC → TAC, GAC→ GAG	3.2
Total		**100**

*Codons within RRDR (Rifampicin resistant determining region), $^{\$}$Codons outer to RRDR, $^{+}$ novel mutations.

was identical to C027. However, they belonged to separate clusters (cluster 2 and 3 respectively) (Figure 3). Further, isolates PC88 and Mo33 containing the novel mutation at codon 184 belonged to, two separates clusters (cluster 2 and 5 respectively) as represented in Figure 3.

Discussion

IS6110 based DNA fingerprinting of *M. tuberculosis* is highly effective in detecting transmission and the disease outbreak [12]. The present study utilized the *IS6110* fingerprinting to determine the genetic diversity of *M. tuberculosis* and rifampicin-resistant *M. tuberculosis* isolates retrieved from primary and secondary pulmonary TB patients in Western province of Sri Lanka. Among the tested *M. tuberculosis* isolates (n = 77), the number of copies of *IS6110* varied from 1 to 14. All isolates had at least one copy and copy numbers 3 and 4 were not observed in any isolates. Most of isolates (73%) had high copy numbers ranging from 6-14 of which 16 (21%) showed 11 copies. Relatively high copy number isolates have been reported in studies from different countries such as USA [13], where 74% isolates had 6-15 copies, and India [14] where 50% of isolates had 6-15 copies. Furthermore, prevalence of high copy number (> 8 copies) isolates are dominant in Asian countries, around 95% in Chinese, Mongolian, Filipino, and Korean isolates, while in the Western Pacific region such as Thailand, Malaysia and Vietnam the prevalence is around 60% [15]. Therefore, it could be concluded that the high copy number is the most prevalent on the Asian continent. Out of all, 13% (n = 10) of isolates had only a single *IS6110* copy which was similar to results in previous studies in Kandy district of the central province (13.6%) and Western province (14.6%) of Sri Lanka [16,17]. In these studies, most of isolates had copy numbers ranging from 1-5 (52% and 68%, respectively). Strains lacking in *IS6110* elements were also included in this category. Therefore, compared with previous studies, the present study showed a reduction trend of isolates with low copy number (< 5 copies, 18.2%). Similar results have been presented in a study from German authors where the prevalence of low copy isolates was 11.9% in 1994 [18] and 2.39% in 1997 [19]. Furtehrmore, authors from Western province of Sri Lanka reported shift of copy numbers from < 5 copies to > 6 [17]. The present study showed particular difference compared to previous studies as the copy numbers 3 and 4 were absent in rifampicin-sensitive isolates while copy number ranging from 2-5 were absent in rifampicin-resistant isolates indicating the emergence of new isolates. Lack of copy numbers 3 and 5 is common in some countries from the Indian ocean region and southern Asiatic regions such as La Reunion, Comoros and Mauritius [20] and has been reported in the Western Pacific region such as Philippines, China and Mongolia [15]. The variation observed in the copy numbers could be due to migrants and travellers, as Sri Lanka is a popular touristic destination. Variation of RFLP pattern of *IS611* copy number in one region emphasizes the necessity of continual conduction of epidemiological screening. The ongoing transmission of *M. tuberculosis* in certain settings is influenced by the genetic families such as Beijing family (Northern China, Japan, and South Korea), Central Asian strains (CAS) family, East African-Indian (EAI) family and Latin American Mediterranean (LAM) family. According to the literature, strains with high *IS6110* copy number, especially ≥ 15, indicate typical Beijing strains [4, 21], while the most of EAI strains have < 5 copies [22]. Strains with copy number 8 - 13 have been classified as Beijing family, K family (a sub-lineage of the Beijing family) or CAS family [23]. Therefore, it is concluded that RFLP results should be combined with results of more precise and informative methods of typing such as spoligotyping or MIRU-VNRT to classify *M. tuberculosis* strains into definitive families. Highly polymorphic RFLP fingerprints of *IS6110* elements

tested in the present study suggests the absence of large-scale outbreak risk. Among the rifampicin-resistant isolates, the 87% (n = 27) were genetically distinct. It supports the assumption that most of rifampicin-resistant *M. tuberculosis* isolates in the Western province do not originated from a subpopulation with the same clonal origin. Furthermore, there was no close relationship or identical fingerprinting patterns between any rifampicin-sensitive and rifampicin-resistant strains. This indicates the absence of similar clonal origination of sensitive and resistant isolates. Recent epidemiological studies shown the presence of identical *IS6110* fingerprint patterns of *M. tuberculosis* strains in transmission [24]. In this study, six clusters with identical fingerprinting patterns were observed. Among them, five clusters were represented only by rifampicin-sensitive isolates while other cluster included rifampicin-resistant isolates (n = 4). Each cluster with the identical fingerprinting pattern consisted of 2-4 isolates suggestive of the presence of mini TB outbreak in Western province of Sri Lanka. Among the fingerprinted rifampicin-resistant isolates, 13 had novel point mutation in codon 626 of *rpoB* gene. It could be concluded that these isolates with the novel mutation do not originate from the outbreak as they revealed 11 distinct fingerprinting patterns distributed in all clusters of the dendrogram. Two isolates with mutation in codon 626 had the same DNA fingerprinting pattern and could present the risk for transmission of this genotype. The limitation of the study are the small sample size and manual analysis of RFLP data. In conclusion, high genetic variation was observed among both, rifampicin-sensitive and -resistant isolates, and there was no strong genetically relatedness between any rifampicin-sensitive and -resistant isolates based on the *IS6110* DNA fingerprinting. There is no risk of large-scale outbreak of drug resistant TB in Western province of Sri Lanka. However, evidences for transmission and mini-outbreak of several genotypes of both, rifampicin sensitive and -resistant *M.* tuberculosis, were found. Further studies on genotypes and transmission of drug-resistance using rapid an less cumbersome DNA based typing methods such as spoligotyping, MIRU-VNRT and whole genome sequencing for a larger number of strains could be performed.

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Authors' contributions

CPA, SSW and JP designed the study; CPA carried out the laboratory work and analyzed the data; CPA, JP and SSW interpreted the data. CPA drafted the manuscript. SSW and JP supervised the work of CPA and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval

The Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka approved the study (ERC Number is EC/06/062) and the informed consent form patients were obtained prior to sample collection.

References

1. World Health Organization report (2015) Global tuberculosis control. Geneva, Switzerland: World Health Organization. Available: http://www.who.int/tb/publications/global_report/en/ Accessed: Accessed 18-November-2016
2. Lisdawati V, Puspandari N, Rifati L, Soekarno T, Melatiwati M, Syamsidar K, Ratnasari L, Izzatun N, Parwati I (2015) Molecular epidemiology study of *Mycobacterium tuberculosis* and its susceptibility to anti-tuberculosis drugs in Indonesia. BMC Infect Dis. 1:366
3. World Health Organization report (2014) Drug-resistant TB surveillance & response; supplement global tuberculosis report 2014. Geneva, Switzerland: World Health Organization. Available: http://www.who.int/tb/publications/global_report/gtbr14_supplement_web_v3.pdf Accessed 18 November 2016
4. Jagielski T, van Ingen J, Rastogi N, DziadekJ, Mazur PK, Bielecki J (2014) Current methods in the molecular typing of *Mycobacterium tuberculosis* and other mycobacteria. BioMed Research International. Available: http://dx.doi.org/10.1155/2014/645802 Accessed: 18 November 2016
5. Cole ST (2002) Comparative and functional genomics of the *Mycobacterium tuberculosis* complex. Microbiology 148: 2919-2928
6. Roychowdhury T, Mandal S, Bhattacharya A. (2015) Analysis of *IS6110* insertion sites provide a glimpse into genome evolution of *Mycobacterium tuberculosis*. Sci Rep 5: 12567
7. De Kantor IN, Kim SJ, Frieden T, Frieden T, Laszlo A, Luelmo F, Norval P-Y, Rieder H, Valenzuela P, Weyer K. (1998) Laboratory services in tuberculosis control: culture part III. Geneva, Switzerland; World Health Organization: Available: http://apps.who.int/iris/bitstream/10665/65942/3/WHO_TB_98.258_(part3).pdf Accessed: 18 November 2016
8. Forbes BA, Banaiee N, Beavis KG, Brown–Elliott BA, Latta PD, Elliott LB (2004) Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis* complex. In Susceptibility testing of Mycobacteria, Nocardiae and other aerobic Actynomycetes approved standard. USA: Clinical and Laboratory Standard Institute. 3-25
9. Warren RM, van Helden PD, Gey van Pittius NC (2009) Insertion element *IS6110*-based restriction fragment length

polymorphism genotyping of *Mycobacterium tuberculosis*. Meth Mol Biol 465: 353-370
10. Adikaram CP, Wijesundera WSS, Perera J (2012) Geographical profile of *rpoB* gene mutations in rifampicin resistant *Mycobacterium tuberculosis* isolates in Sri Lanka. Microbial Drug Res 5: 525-530
11. Phyfi FJ (2006) Fast and easy online creation and manipulation of phylogeny color figures. BMC Bioinformatics 7: 315-322
12. Sougakoff W (2011) Molecular epidemiology of multidrug-resistant strains of *Mycobacterium tuberculosis.* Clin Microbiol Infec 17: 800-805
13. Yang Z, Barnes PF, Bhaves F, Eisenach KD, Weis SE, Bates JH, Cave MD (1998) Diversity of DNA Fingerprints of Mycobacterium tuberculosis Isolates in the United States. J Clin Microbiol 38: 191–197
14. Bhanu NV, van Soolingen D, van Embden JD, Dar L, Pandey RM, Seth P (2002) Predominance of a novel *Mycobacterium tuberculosis* genotype in the Delhi region of India. Tuberculosis (Edinb) 82: 105-112
15. Park YK, Bai GH, Kim SJ (2000) Restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolated from countries in the Western Pacific Region. J Clin Microbiol 38: 191-197
16. Magana-Arachchi DN, Medagedara D, Thevanesam V (2011) Molecular characterization of *Mycobacterium tuberculosis* isolates from Kandy, Sri Lanka. Asian Pac J Trop Dis 3: 181-186
17. Magana-Arachchi DN, Perera AJ, Senaratne V, Chandrasekharan NV (2010) Pattern of drug resistant and RFLP analysis of *Mycobacterium tuberculosis* strains isolated from recurrent tuberculosis patients in Sri Lanka. South East Asian J top Med public health 41: 583-590
18. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, Schecter GF, Daley CL, Schoolnik GK (1994) The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. N Engl J Med 330: 1703-1709
19. Niemann S, Rusch-Gerdes S, Richter E (1995) *IS6110* fingerprinting of drug-resistant *Mycobacterium tuberculosis* strains isolated in Germany during 1995. J Clin Microbiol 35: 3015–3020
20. Das SD, Narayanan S, Hari L, Hoti SL, Thangathurai RK, Charles N, Jaggarajamma K, Narayanan PR (2005) Differentiation of highly prevalent *IS6110* single-copy strains of Mycobacterium tuberculosis from a rural community in South India with an ongoing DOTS programme. Infect Genet Evol 5: 67-77
21. Alonso H, Samper S, Martín C, Otal I (2013) Mapping *IS6110* in high-copy number *Mycobacterium tuberculosis* strains shows specific insertion points in the Beijing genotype. BMC Genomics 14: 422
22. Buu TN, van Soolingen D, Huyen MN, Lan NT, Quy HT, Tiemersma EW, Kremer K, Borgdorff MW, Cobelens FG (2012) Increased transmission of *Mycobacterium tuberculosis* Beijing genotype strains associated with resistance to streptomycin: a population-based study. PLoS One **7**: e42323
23. Choi GE, Jang MH, Song EJ, Jeong SH, Kim JS, Lee WG, Uh Y, Roh KH, Lee HS, Shin JH, Ryoo NH, Kim YR, Jeong J, Kim JH, Lee SM, YI J, Hwang SH, KimHH, Lee EY, Chang CL, Kim MB, Kim YD (2010) *IS6110*-Restriction fragment length polymorphism and spoligotyping analysis of *Mycobacterium tuberculosis* clinical isolates for investigating epidemiologic distribution in Korea. J Korean Med Sci 25: 1716-1721
24. Green E, Obi LC, Okoh AI, Nchabeleng M, deVilliers BE, Letsoalo T, Hoosen AA, Bessong PO, Ndip RN (2013) *IS6110* Restriction fragment length polymorphism typing of drug-resistant *Mycobacterium tuberculosis* strains from Northeast South Africa. J Health Popul Nutr 31: 1-10

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